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Effect of sowing density on performance and biometric features of pedunculate oak

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Abstract

Sowing density is one of the most important factors influencing seedling quality. In forest nurseries, to ensure full seeding, it is recommended to obtain approximately 200 seedlings from 1 m^2 (ZHL 2012). The aim of the study was to assess the effect of sowing density on sowing efficiency and the morphological features of pedunculate oak seedlings. Acorns were sown directly by broadcast seeding on 20 test plots, each 4 m in length, in four replications. The following variants were used:
- 250 acorns capable of germination totaling 341 acorns per 1 linear meter (0.9 m^2);
- 300 acorns capable of germination totaling 410 acorns per 1 linear meter (0.9 m^2);
- 350 acorns capable of germination totaling 478 acorns per 1 linear meter (0.9 m^2);
- 400 acorns capable of germination totaling 546 acorns per 1 linear meter (0.9 m^2);
- 450 acorns capable of germination totaling 614 acorns per 1 linear meter (0.9 m^2).

At the end of the growing season, seedlings were counted per 1 m (0.9 m^2) in the central part of each plot. Sowing efficiency was determined as a percentage of seedling yield from the sown seeds. For laboratory analysis, 30 seedlings from each plot (600 in total) were collected. Based on biometric features like shoot height, root collar diameter, and dry weights of shoots, roots, and leaves, quality indicators such as the sturdiness quotient (SQ) and the Dickson quality index (DQI) were calculated.

Sowing efficiency at the end of the vegetation season ranged between 39.68% and 50.12% and was not statistically significant. The research revealed that sowing density influenced seedling biometric characteristics. Pearson's correlation coefficients showed significant negative correlations between the number of seedlings obtained in an experimental plot and the dry weight of the root, number and dry mass of leaves, as well as the DQI. The seedlings from all sowing variants complied with the binding Polish standard, outlined in the Regulation of the Minister for the Environment of 18 February 2004 (Ministerstwo Środowiska 2004) by over 96%.

Keywords: seeding density; sowing rate; seedling quality; sowing performance; sturdiness quotient (SQ); Dickson quality index (DQI); *Quercus robur* L.

Introduction

Oaks hold a significant economic and ecological role in the forests of the northern hemisphere (Johnson et al. 2019). Specifically in Europe, pedunculate oaks (*Quercus robur* L.) and sessile oaks (*Quercus petraea* (Matt.) Liebl.) are the most important woody species (Gil-Pelegrín et al. 2017). There is a prevailing belief that climate change will increase the prevalence of oak in central Europe (Perkins et al. 2018). Across European forests, Poland included, there is a notable shift in tree stands to accommodate changing habitat conditions. Consequently, the share and role of deciduous species, especially pedunculate oak and sessile oak, is on the rise (Andrzejczyk 2009).

Artificial regeneration can be carried out by direct sowing of acorns or the transplantation of nursery-grown seedlings. Intriguingly, the survival rates of seedlings produced directly from planted acorns are often lower than those of seedlings grown in nurseries (González-Rodríguez et al. 2011). As a result, artificial regeneration, specifically using nursery-grown seedlings, is the preferred method for establishing oak cultures (Knoot et al. 2010, Villar-Salvador et al. 2013). Presently, oaks constitute 7.7% of Polish forests (Rozkrut 2022). The pedunculate oak in Poland has a fruiting pattern where abundant fruit is produced every 3–8 years (Suszka 1994), and mast years can be as infrequent as once every nine years (Kantorowicz 2000). Acorns remain viable for a short time, which means that their storage is limited. The challenges extend to the high moisture content in acorns and their improper postharvest treatment may prevent their successful storage. The success of silviculture depends on nursery practices because they have a significant impact on the size and vigour of seedlings. The production method of the plant material has a huge impact on its behaviour in the crop (Cicek et al. 2007). Factors within nursery production are pivotal in deciding seedling quality (Wesoły and Hauke 2009). In Poland, the production of bare-root seedlings is predominantly undertaken in traditional field nurseries.

In nurseries, sowing density, and the resulting seedling density, are one of the most important factors affecting the quality of plant material. It depends mainly on seed properties and the sowing method. By ensuring the correct sowing rate, one sets the stage for optimal plant establishment, which in turn shapes factors like light conditions, water supply, and nutrient availability. Errors in establishing the ideal sowing density may result in reduced sowing efficiency and inferior seedling parameters (Wesoły and Hauke 2009). In forest nurseries, for comprehensive seeding under-regulated greenhouse conditions, it is advised to aim for approximately 200 seedlings from a 1 m^2 area (ZHL 2012). Unfortunately, sowing standards for oaks in the bare-root seedling production system might not be optimized due to a lack of studies on the subject. The purpose of this study was to assess the effect of seedling density on both the efficiency and morphological features of oak seedlings. Different seedling densities were obtained by experimenting with varying amounts of sown acorns.

Material and methods

Acorns for the experiment were collected on November 1–2, 2017, from the Złotoryja Forest District, compartment 269a (N: 50.986, E: 15.925). They exhibited a germination capacity of 73.24%, i.e. there were 167 germinable acorns per 1 kg. These acorns had a 1000 seed weight of 4.376 kg. On November 17, 2017, the acorns were sown at the forest nursery located in Wojcieszów Dolny (N: 50.978, E: 15.915).

The experimental plot consisted of two nursery seedbeds, each 40 m in length and 0.9 m in width. These seedbeds were divided into 20 test plots, each 4 m in length. For each density variant, four repetitions were executed using the direct broadcast seeding method. The following variants were used:

- 250 acorns capable of germination totaling 341 acorns per 1 linear meter (0.9 m^2);
- 300 acorns capable of germination totaling 410 acorns per 1 linear meter (0.9 m^2);
- 350 acorns capable of germination totaling 478 acorns per 1 linear meter (0.9 m^2);
- 400 acorns capable of germination totaling 546 acorns per 1 linear meter (0.9 m^2);
- 450 acorns capable of germination totaling 614 acorns per 1 linear meter (0.9 m^2).

Seeds were covered with sand and secured with a sheet of raschel fabric. As a part of ongoing maintenance, manual weeding was performed, and the seedbeds were chemically treated against oak powdery mildew, with both activities carried out thrice, in three sets of repetitions. On three specific dates in 2018 – May 7, July 6, and September 27 – seedlings were counted per 1 m in the central section of each plot, specifically 1.5 m from both ends. Using the obtained data, sowing efficiency was calculated. This metric expressed the percentage of seedlings that emerged from the sown acorns.

Additionally, once the growing season concluded, 30 seedlings were collected from the central part of the experimental plot for laboratory analyses. This resulted in a total of 600 plants. Subsequent analyses involved measuring the shoot length and root-collar diameter (to a precision of 1 and 0.1 mm, respectively). Furthermore, the total leaf count on each shoot was documented, and the dry weight (60°C for 48 h) of the shoot, root, and leaves was ascertained (to an accuracy of 0.01 g).

Employing these biometric features, several indicators determining seedling quality were calculated, viz. the sturdiness quotient (SQ) (Jaenicke 1999) and the Dickson Quality Index (DQI) (Dickson et al. 1960). The percentage of seedlings aligns with the criteria defined in the Regulation of the Minister for the Environment (Ministerstwo Środowiska 2004). In accordance with this regulation, the specifications for 1-year-old pedunculate oak planting material stipulate a minimum height of 7 cm and a root collar diameter of at least 2 mm.

$$\mathrm{SQ} = \frac{\text{Height [cm]}}{\text{Root collar diameter [mm]}} \quad [1]$$

$$\mathrm{DQI} = \frac{\text{Seedling dry weight [g]}}{\frac{\text{Height [cm]}}{\text{Root collar diameter [mm]}} + \frac{\text{Shoot dry weight with leaves [g]}}{\text{Root dry weight [g]}}} \quad [2]$$

Statistical analyses were performed using the Statistica 13.3 software package (TIBCO Software 2017). Techniques such as one-way analyses of variance (ANOVA) and linear regression were utilized for the analyses. A p-value < 0.05 was considered indicative of statistically significant differences between mean values. To assess variations in seedling features, ANOVA was performed. Subsequent to this, Tukey's a posteriori test was implemented, with a global $a = 0.05$ (a, significance level). Lastly, the significance of Pearson's coefficient (r) was statistically significant at $p < 0.05$.

Results

The efficiency of sowing at the culmination of the vegetation period fluctuated between 39.68% and 50.12% (Table 1). The conducted experiment did not demonstrate a significant impact of sowing density on production efficiency ($p = 0.104$). Notably, there was a 10% difference in sowing capacity between the least effective variant (variant 5) and the most effective one (variant 4). When comparing these two variants, the results approached statistical significance ($p = 0.052$). Figure 1, which showcases Pearson's correlation coefficient, highlighted a notable positive correlation between the sowing variant and the resultant number of seedlings in each experimental plot ($r = 0.9210$, $p = 0.02$).

The average shoot length ranged from 14.5 to 18.0 cm (Table 2). Interestingly, variant 4, which showcased the

Table 1. The number of seedlings obtained per 1 m (± standard deviation) and sowing performance at the end of the growing season and mean sowing efficiency

Number of acorns capable of germination (Variant)	Mean number of seedlings obtained in 1 mb (0,9 m^2)			Sowing efficiency (%)		
Date of measurement	7.05	6.07	27.09	7.05	6.07	27.09
250 (1)	67.25 ± 15.69 a	112.50 ± 14.08 a	113 ± 13.93 a	26.9 ± 6.27 a	45.0 ± 5.63 a	45.5 ± 25.57 a
300 (2)	80.75 ± 19.93 ab	131.50 ± 18.35 ab	135 ± 20.31 ab	26.91 ± 6.64 a	43.83 ± 6.11 a	45.00 ± 6.76 a
350 (3)	104.25 ± 9.95 abc	150.75 ± 9.91 bc	154 ± 11.32 bc	29.78 ± 5.70 a	43.07 ± 2.83 a	44.07 ± 3.23 a
400 (4)	116.25 ± 9.46 bc	178.75 ± 14.10 cd	200 ± 12.36 d	31.62 ± 2.36 a	49.06 ± 3.52 a	50.12 ± 3.09 a
450 (5)	126.50 ± 20.03 c	196.25 ± 20.70 d	183 ± 20.70 cd	25.83 ± 4.45 a	39.72 ± 4.8 a	39.68 ± 4.6 a

Notes: a–d – homogeneous groups determined by Tukey's test, $p < 0.05$.

Table 2. Mean values (± standard deviation) of traits of oak seedlings grown in different sowing rate variants

Number of acorns capable of germination (Variant)	Shoot height (cm)	Root collar diameter (mm)	Shoot dry weight (g)	Root dry weight (g)	Number of leaves (pcs)	Leaf dry weight (g)
250 (1)	15.4 ± 6.56 ab	0.48 ± 0.13 ab	1.00 ± 0.75 ab	3.23 ± 2.09 a	13 ± 8.54 a	1.30 ± 0.83 a
300 (2)	17.9 ± 8.76 a	0.51 ± 0.14 a	1.28 ± 1.12 a	3.35 ± 2.07 a	12 ± 7.81 ab	1.02 ± 0.60 ab
350 (3)	18.0 ± 10.17 a	0.49 ± 0.13 a	1.20 ± 1.06 a	3.03 ± 1.99 ab	11 ± 8.06 ab	1.13 ± 1.00 ab
400 (4)	14.5 ± 5.77 b	0.44 ± 0.12 b	0.80 ± 0.61 b	2.47 ± 1.75 b	8 ± 6.17 b	0.66 ± 0.67 b
450 (5)	17.3 ± 8.29 ab	0.47 ± 0.13 ab	1.07 ± 0.99 ab	2.86 ± 2.03 ab	9 ± 6.47 ab	0.91 ± 0.80 ab

Notes: a–b – homogeneous groups determined by Tukey's test, $p < 0.05$.

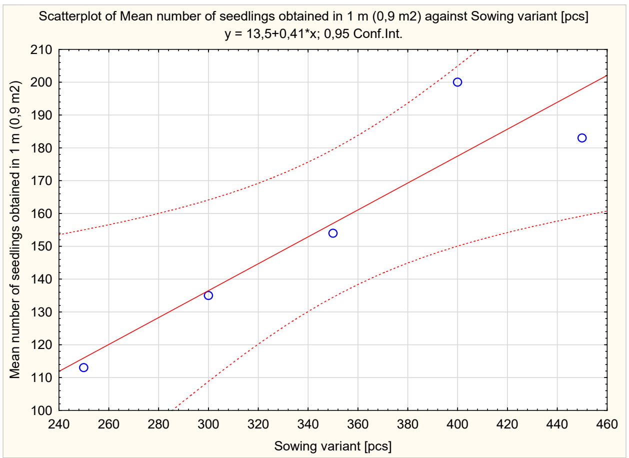

Figure 1. Scatterplot showing the relationship between sowing variant and average number of seedlings obtained per 1 m (0.9 m2)

highest sowing efficiency, was characterized by having the shortest average shoot length (Table 2). Furthermore, seedlings stemming from variant 4 typically exhibited a dry shoot weight of under 1 g (Table 2). Table 3 confirms that shoot dimensions, whether represented through height or dry weight, were not significantly influenced by the seedling density present within the experimental plots. Moreover, the smallest root system, delineated by metrics such as root collar diameter and dry root mass, was obtained for variant 4 – the variant with the highest seedling density per unit area (Table 2). A discernible negative correlation exists between the number of procured seedlings and the size of their root systems when described in terms of dry weight (Table 3). Additionally, an increment in seedling density correlated with a reduced average leaf count and decreased leaf weight (Table 3).

Table 3. Pearson's coefficients of correlation between number of seedlings obtained and seedling characteristics

Seedling feature	R	P-value
Shoot height	–0.23	0.704
Shoot dry weight	–0.51	0.373
Root collar diameter	–0.73	0.159
Root dry weight	–0.91	0.030 *
Number of leaves	–0.99	< 0.001 **
Leaf dry weight	–0.91	0.028 *
SQ	–0.595	0.429
DQI	–0.97	0.003 **

Notes: * Correlation is significant at the 0.05 level. ** Correlation is significant at the 0.01 level.

In the next stage, the sturdiness quotient (SQ) was calculated [variant 1]. As outlined in Table 2, the average SQ values exhibited minor differences, ranging between 3.20 and 3.64. Seedlings grown at the lowest density, i.e., variant 1, demonstrated the lowest sturdiness. However, the analysis of variance disclosed disparities across the variants, yet the sowing density was determined to not influence SQ. Pearson's correlation coefficient (Table 3), revealed no significant correlations between SQ and the total number of seedlings derived from each experimental plot. DQI [2] showcased variability contingent upon the quantity of seedlings obtained (Table 3). The lowest value of 0.11 was recorded for variant 4, which was characterized by the highest sowing efficiency (Table 4). Furthermore, a mere 0.83% to 3.33% of seedlings sourced from every sowing variant did not fulfil the stipulated criteria for minimum height and root collar diameter, as delineated by the prevailing Polish standard (Table 4).

Table 4. Mean values (± standard deviation) of seedling quality indicators for the analysed sowing densities

Number of acorns capable of germination (Variant)	SQ	DQI	Regulation of the Environment Minister of February 18, 2004	
			Shoot height minimum 7 cm	Root collar diameter minimum 2 mm
250 (1)	3.20 ± 0.96 b	1.69 ± 1.25 a	99.17	100
300 (2)	3.46 ± 1.06 ab	1.56 ± 0.95 a	98.27	100
350 (3)	3.63 ± 1.33 a	1.40 ± 1.03 ab	99.17	100
400 (4)	3.37 ± 1.05 ab	1.13 ± 0.94 b	96.67	98.58
450 (5)	3.64 ± 1.13 a	1.34 ± 1.17 ab	99.17	99.17

Notes: a–b – homogeneous groups determined via using Tukey's test, $p < 0.05$.

Discussion

In the case of oaks, uneven emergence is a significant problem, reducing sowing efficiency in comparison to the evaluation of seed sowing value. We typically observe the first seedlings emerging around 2 weeks after sowing, with the last ones appearing 16–17 weeks later (Andrzejczyk 2009). This study also noted uneven seedling emergence (Table 1). The seed coat suppresses germination, as it hinders the flow of water and air into the acorn. In container forest nurseries, scarification is utilized to accelerate acorn germination and standardize seed emergence (Skrzyszewska et al. 2019). Due to this uneven germination, there is a variance in seedling growth, affecting their quality. Under laboratory conditions, emergence rates for pedunculate and sessile oak are around 60% (Tylkowski and Bujarska-Borkowska 2011). In field nurseries, acorns might be sown in the fall, but they are then vulnerable to being eaten by animals. In addition, unfavourable atmospheric conditions occurring in winter, like excessive moisture or low temperatures, or premature emergence in the spring can negatively influence seedling emergence. The repetition of early emergence in the spring has an adverse effect on seedling emergence. Nursery production favours faster-growing seedlings, which puts slower growers at a competitive disadvantage. Late-germinating acorns struggle for light access due to the leaves from earlier sprouted seeds overshadowing them. An insufficient amount and/or inadequate spectral composition of light can result in reduced plant growth (Starck et al. 1993). Deciduous seedlings can absorb water and nutrients through their leaves (Landis et al. 1989), and high planting densities are not favourable for this function. In the present experiment, the effect of sowing density on germination efficiency was not shown. The relationship between sowing density and germination in forest nurseries is complex and may be influenced by various factors. The absence of statistical significance is likely due to the utilization of seeds with similar viability and proper sowing density.

The quality of the nursery material is mainly determined by nursery cultural practices. It is known that sowing density affects the size and vigour of seedlings (Devetaković et al. 2020), which in turn may determine the success rate after transplanting in the forest culture. Sowing density significantly influences a notable effect of sowing density is on the biometric attributes of seedlings. Decreasing plant density tends to enhance their nutritional status (Güner et al. 2016). Evaluating morphological traits is instrumental in predicting seedling survival in cultivation. Numerous studies consistently emphasize the significance of assessing morphological traits when predicting the survival of seedlings in cultivation. Tsakaldimi et al. (2012) demonstrated that the initial morphological characteristics of seedlings play a crucial role in predicting the field performance of Mediterranean species. Key indicators such as root-collar diameter, total dry weight, and Dickson's quality index were identified as particularly influential. Additionally, Pinto et al. (2011) and Haase (2007) underscore the importance of both morphological and physiological evaluations in determining seedling quality and subsequent field performance.

Seedling height is a significant metric, particularly in a cultivation context. The competition for light, both among seedlings and with understory vegetation, can restrict their growth. In the case of intense competition, taller seedlings are favoured due to their superior survival and growth rates (Mohammed et al. 1998, South and Mitchell 1999). While tall seedlings thrive in optimal environmental conditions, smaller seedlings tend to fare better in less favourable settings (Grossnickle and MacDonald 2018). Various studies highlight that taller seedlings result from reduced numbers of seedlings per 1 m^2, as evidenced in species like *Quercus robur* (Wesoły et al. 2017), *Cedrus libani* (Güner et al. 2016), *Fraxinus angustifolia*, *Picea glauca*, *Pseudotsuga menzii*, and *Pinus resinosa* (Cicek et al. 2007). However, contrasting findings suggest that for *Quercus alba* seedlings, density does not influence their height (Wichman and Coggeshall 1983). Our research found no statistically significant correlation between the density of seedlings and their height.

The quality of a seedling is intrinsically linked to the parameters of its root system. A well-developed, functional root system is required for forest regeneration success (Grossnickle and MacDonald 2018). Root collar diameter and root system mass are commonly used metrics to describe root characteristics. A seedling with a larger root collar diameter is indicative of a more substantial root system (Ritchie 1984, Duryea and Dougherty 1991). Root collar diameter is deemed a critical trait in seedlings, contributing to their enhanced survival rates (Jaenicke 1999). One-year-old oak seedlings cultivated at higher densities typically have a reduced root collar diameter (Wichman and Coggeshall 1983, Wesoły et al. 2017). This research reveals a strong and statistically significant correlation between seedling dry weight and density ($r = -0.9$, $p = 0.03$). However, the correlation with root collar diameter is not statistically significant. A more extensive root mass suggests a heightened ability to absorb water and nutrients shortly after replanting, which augments the chances of mitigating planting stress (Tsakaldimi et al. 2012). In addition, the size of the root system has implications for the continued growth of seedlings. A study on 10-year-old Douglas fir trees found that the initial root system size correlated with increases in both breast height diameter and root biomass (Sundström and Keane 1999). *Quercus falcata* seedlings grown at a density of 100 seedlings per 1 m^2 exhibited significant differences in both survival rate and diameter growth during their second year in forest cultivation compared to those cultivated at higher densities (Howell and Harrington 1998).

Numerous studies addressing seedling quality apply indicators rooted in the biometric attributes of seedlings. The SQ is defined as the height-to-thickness ratio of the seedling shoot. A high SQ indicates that seedlings are slender and possibly weak, whereas a low SQ suggests sturdiness. Seedlings with a lower SQ tend to be more resilient against adverse abiotic factors like drought and wind, often resulting in enhanced survival in cultivation, especially in dry places (Jaenicke 1999, Ivetić et al. 2016, Grossnickle and MacDonald 2018). In the provided study, the sturdiness quotient ranged from 3.2 to 3.63. An ideal SQ for a sturdy seedling is below six (Jaenicke 1999). Our findings fall within these recommended ranges, indicating that the seedlings, irrespective of sowing density, an adequate sturdiness quotient. Studies suggest seedlings grown at lower densities have a reduced SQ, indicating a more balanced growth (Dierauf and Garner 1996, Batziou et al. 2016); however, our results did not confirm this.

Another indicator often used for seedling quality assessment is the DQI (Dickson et al. 1960). The DQI can serve as a reliable predictor for seedling survival in cultivation under water-scarce conditions (Bayala et al. 2009). Optimal phenotypes possess a higher DQI value, reflecting seedling vigour and a balanced distribution of seedling biomass (Scalon et al. 2014). In our research, the lowest DQI was observed for seedlings grown at maximum density. Additionally, there was a robust and statistically significant correlation ($r = -0.9$, $p = 0.003$) between seedling density and DQI. Importantly, environmental and genetic factors can potentially influence the indicators used to determine seedling quality and survivability (Pinto et al. 2011).

Conclusion

The conducted study revealed that sowing density does not significantly influence the efficiency of nursery production. However, there was observed variability in seedlings concerning their biometric characteristics. It is especially important to emphasize the development of the root mass, as it is pivotal for the subsequent growth and establishment of seedlings in cultivation. Excluding the data derived from the sowing density of 400 acorns per 0.9 m^2, most of the outcomes are statistically not significant. This suggests that the sowing density of acorns can potentially be decreased, benefitting the quality of the produced. Based on the above results, we recommend direct sowing, in the field nursery, of 420–430 acorns capable of germination per 1 m^2. This creates a potential advantage in the growth of these seedlings and may result in greater cultivation success.

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